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IN THE UNITED STATES PATENT AND  
TRADEMARK OFFICE

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In Re the Application of: George Seidel, Lisa Herickhoff, John Schenk

Serial Number: New: \_\_\_\_\_ (Parent: 09/448,643)

Filed: New: \_\_\_\_\_ (Parent: 11/24/1999)

New Title: Multiple Sexed Embryo Production System for Mammals Using Low Numbers of Spermatozoa

Parent Title: Multiple Sexed Embryo Production System for Mammals

Group Art Unit: New: \_\_\_\_\_ (Parent: 1655)

Examiner: New: \_\_\_\_\_ (Parent: Carla J. Myers)

Assignee: XY, Inc. and Colorado State University through its agent Colorado State University Research Foundation

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**FIRST PRELIMINARY AMENDMENT**

Before examining the above-referenced application and calculating the fee due, please amend the application as follows.

In the specification:

Kindly change the title to read: --Multiple Sexed Embryo Production System for Mammals Using Low Numbers of Spermatozoa--.

Please insert the following language as the first paragraph of the specification:

-- This application is a continuation of United States Application No. 09/448,643, filed November 24, 1999, now issued as United States Patent No. \_\_\_\_\_ which was a continuation of United States Application No. 09/015,454, filed January 29, 1998, now issued as United States Patent No. 6,071,689 which was a continuation - in - part of United

States Application No. 09/001,394, filed December 31, 1997, now issued as United States Patent No. 6,149,867, each hereby incorporated by reference.--

In the claims:

Please cancel claims: 1-123 and 142-143.

Pursuant to 37 C.F.R. §1.121, the applicant submits a clean set of claims as amended.

The applicant has consolidated all separate amendments to the claims into a single clean version which is to be construed as a cancellation of all previous versions of the claims with respect to this application. The applicant respectfully requests entry of the clean version of the claims as set forth beginning on the next separate page:

124. A method of producing multiple embryos from a female mammal comprising:
- creating superovulation in said mammal to create at least two eggs comprising the step of using an ovulatory pharmaceutical to cause multiple eggs to be produced;
  - establishing an insemination sample having a low number of sperm cells relative to a typical insemination sample;
  - inserting at least a portion of said insemination sample having a low number of sperm cells into a uterus of said female mammal after onset of estrus; and
  - fertilizing a plurality of said eggs at success levels statistically comparable to a typical insemination dosage;
  - producing at least two embryos from fertilizing said plurality of said eggs in said female mammal.
125. A method of producing multiple embryos according to claim 124 wherein said creating superovulation is encouraged during the estrous cycle.
126. A method of producing multiple embryos according to claim 125 wherein said step of using an ovulatory pharmaceutical comprises the step of injecting said ovulatory pharmaceutical in half day increments between any of days 2 and 18.
127. A method of producing multiple embryos as described in claim 126 wherein injecting said ovulatory pharmaceutical in half day increments comprises injecting at least seven injections and wherein incorporating said estrus manipulation system occurs at least on about the sixth and seventh injections.
128. A method of producing multiple embryos as described in claim 127 wherein inserting at least a portion of said insemination sample having a low number of sperm cells into said uterus comprises inserting said sperm cells into both uterine horns of said uterus.

129. A method of producing multiple embryos as described in claim 128 wherein inserting said sperm cells into both uterine horns comprises inserting said sperm cells approximately between 20 to 24 hours inclusive after said onset of said estrus.
130. A method of producing multiple embryos as described in claim 124 wherein said step of using an ovulatory pharmaceutical to cause multiple eggs to be produced comprises the step of injecting a dosage of follicle stimulating hormone a plurality of times a day.
131. A method of producing multiple embryos as described in claim 130 wherein said step of creating superovulation in said mammal to create at least two eggs further comprises the step of incorporating an estrus manipulation system comprising the step of supplementing said dosage of follicle stimulant hormone with prostaglandin F-2-alpha.
132. A method of producing multiple embryos as described in claim 131 wherein injecting said dosage of follicle stimulating hormone a plurality of times a day comprises injecting said follicle stimulating hormone in approximately half day increments at a dosage level of 6, 6, 4, 4, 2, 2, 2, and 2 mg between days 9 and 12 inclusive of the estrous cycle and wherein supplementing said dosage of follicle stimulant hormone with prostaglandin F-2-alpha comprises supplementing 25 and 12.5 mg of prostaglandin F-2-alpha on the sixth and seventh dosages, respectively, of said follicle stimulating hormone.
133. A method of producing multiple embryos as described in claim 124 and further comprising the step of separating sperm cells based on the amount of nuclear DNA each said sperm cell contains
134. A method of producing multiple embryos as described in claim 133, further comprising the step of staining said nuclear DNA of a plurality of said sperm cells.

135. A method of producing multiple embryos as described in claim 134, wherein said step of separating said sperm cells comprises sorting said sperm cells using a flow cytometer.
136. A method of producing an animal of a desired sex in accordance with the process as described in claim 135.
137. A method of producing an animal of a desired sex in accordance with the process as described in claim 136 further comprising sorting said sperm cells at a rate greater than 500 sorts per second .
138. A method of producing an animal of a desired sex as described in claim 136 further comprising sorting said sperm cells at a rate greater than 2000 sorts per second.
139. A method of producing an animal of a desired sex as described in claim 136 further comprising chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment comprising establishing a sheath fluid which contains about 2.9% sodium citrate.
140. A method of producing an animal of a desired sex as described in claim 139 wherein chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment comprises establishing a sheath fluid which contains a herpes buffered medium.
141. A method of producing an animal of a desired sex as described in claim 140 further comprising collecting cells having the desired characteristic and cushioning said cells from impact with a collection container which has a wide opening.

## REMARKS

This application is a continuation of United States Application No. 09/448,643, filed November 24, 1999, now issued as United States Patent No. \_\_\_\_\_ which was a continuation of United States Application No. 09/015,454, filed January 29, 1998, now issued as United States Patent No. 6,071,689 which was a continuation - in - part of United States Application No. 09/001,394, filed December 31, 1997, now issued as United States Patent No. 6,149,867. The title of this continuation application has also been amended to distinguish the invention from the prior cases.

Claims 1-123 and 142 have been canceled without prejudice. The applicant reserves the right to have these claims examined as originally recited without any reduction in breadth as part of a subsequent continuation, continuation-in-part, continued prosecution, or division application, or the like.

The applicant submits only claims 124-141 as amended above for consideration and respectfully request entry and examination of such claims.

Enclosed with this filing is an Information Disclosure Statement citing the references from the parent case. In addition, references not previously considered by the examiner in the parent application are listed and copies of those additional references are submitted.

A version of the claims with markings to show changes made is provided starting on the next separate page:

VERSION WITH MARKINGS TO SHOW CHANGES MADE

124. A method of producing multiple [, sexed] embryos from a female mammal comprising:
- a. creating superovulation in said mammal to create at least two eggs comprising the step of using an ovulatory pharmaceutical to cause multiple eggs to be produced;
  - [b. determining a sex of a sperm cell of a male mammal;
  - c. sorting according to said sex of said sperm cells]
  - b. establishing an insemination sample having a low number of sperm cells relative to a typical insemination sample;
  - [d] c. inserting at least a portion of said [sorted sperm cells] insemination sample having a low number of sperm cells into [said] a uterus of said female mammal after [an] onset of estrus; and
  - [e] d. fertilizing a plurality of said eggs at success levels statistically comparable to a typical insemination dosage;
  - e. [to] produc[e] ing at least [one animal] two embryos [of the desired sex] from said female mammal.
125. A method of producing multiple [, sexed] embryos according to claim 124 wherein said creating superovulation is encouraged during the estrous cycle.
126. A method of producing multiple [, sexed] embryos according to claim 125 wherein said step of using a an ovulatory pharmaceutical comprises the step of injecting said ovulatory pharmaceutical in half days increments between any of days 2 and 18.
127. A method of producing multiple [, sexed] embryos as described in claim 126 wherein injecting said ovulatory pharmaceutical in half day increments comprises injecting at least seven injections and wherein incorporating said estrus manipulation system occurs at least on about the sixth and seventh injections.

128. A method of producing multiple [, sexed] embryos as described in claim 127 wherein inserting at least a portion of said [sorted sperm cells] insemination sample having a low number of sperm cells into said uterus comprises inserting said sperm cells into both uterine horns of said uterus.
129. A method of producing multiple [, sexed] embryos as described in claim 128 wherein inserting said sperm cells into both uterine horns comprises inserting said sperm cells approximately between 20 to 24 hours inclusive after said onset of said estrus.
130. A method of producing multiple [, sexed] embryos as described in claim 124 wherein said step of using an ovulatory pharmaceutical to cause multiple eggs to be produced comprises the step of injecting a dosage of follicle stimulating hormone a plurality of times a day.
131. A method of producing multiple [, sexed] embryos as described in claim 130 wherein said step of creating superovulation in said mammal to create at least two eggs further comprises the step of incorporating an estrus manipulation system comprising the step of supplementing said dosage of follicle stimulant hormone with prostaglandin F-2-alpha.
132. A method of producing multiple [, sexed] embryos as described in claim 131 wherein injecting said dosage of follicle stimulating hormone a plurality of times a day comprises injecting said follicle stimulating hormone in approximately half day increments at a dosage level of 6, 6, 4, 4, 2, 2, 2, and 2 mg between days 9 and 12 inclusive of the estrous cycle and wherein supplementing said dosage of follicle stimulant hormone with prostaglandin F-2-alpha comprises supplementing 25 and 12.5 mg of prostaglandin F-2-alpha on the sixth and seventh dosages, respectively, of said follicle stimulating hormone.
133. A method of producing multiple [, sexed] embryos as described in claim 124 and further comprising the step[s] of[:]  
[a.] [staining sperm cells of a male mammal;]



- [b.] [sorting] separating sperm cells based on the amount of nuclear DNA each said sperm cell contains [according to said sex of said sperm cells comprises through the use of high speed flow cytometry; and
- c. concentrating said sorted sperm cells].
134. A method of producing multiple [, sexed] embryos as described in claim 1[24] 33, further comprising the step of staining said nuclear DNA of a plurality of said sperm cells [ wherein inserting at least a portion of said sorted sperm cells comprises using a low dose of said sperm cells].
135. A method of producing multiple [, sexed] embryos as described in claim 13[3] 4, wherein said step of separating said sperm cells comprises sorting said sperm cells using a flow cytometer [inserting at least a portion of said sorted sperm cells comprises using a low dose of said sperm cells].
136. A method of producing an animal of a desired sex [comprising producing said animal using] in accordance with the process[es] as described in claim [124]135.
137. A method of producing an animal of a desired sex [comprising producing a sexed sperm cells specimen using] in accordance with the process[es] as described in [of] claim 136 further comprising sorting said sperm cells at [a high speed] a rate greater than 500 sorts per second .
138. A method of producing an animal of a desired sex as described in claim 136 further comprising [inseminating said animal using a low dose of said sperm cells] sorting said sperm cells at a rate greater than 2000 sorts per second.
139. A method of producing an animal of a desired sex as described in claim 136 further comprising chemically coordinating a sheath fluid to create a sheath fluid environment for

said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment comprising establishing a sheath fluid which contains about 2.9% sodium citrate.

140. A method of producing an animal of a desired sex as described in claim 139 wherein chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment comprises establishing a sheath fluid which contains a [herpes] hepes buffered medium.
141. A method of producing an animal of a desired sex as described in claim 140 further comprising collecting cells having the desired characteristic and cushioning said cells from impact with a collection container which has a wide opening.

## CONCLUSION

Claims 1-123 and 142-143 have been canceled without prejudice. Claims 124-141 remain in the application for consideration. The applicant respectfully requests entry of the claims as amended and examination at the Examiner's earliest convenience.

Dated this 20 day of February, 2002.

Respectfully Submitted,  
SANTANGELO LAW OFFICES, P.C.

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